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# Both positive and negative effects on immune responses by expression of a second class II MHC molecule

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ABSTRACT

It is perplexing why vertebrates express a limited number of major histocompatibility complex (MHC) molecules when theoretically, having a greater repertoire of MHC molecules would increase the number of epitopes presented, thereby enhancing thymic selection and T cell response to pathogens. It is possible that any positive effects would either be neutralized or outweighed by negative selection restricting the T cell repertoire. We hypothesize that the limit on MHC number is due to negative consequences arising from expressing additional MHC. We compared T cell responses between B6 mice (I-$A^+$) and B6.$E^+$ mice (I-$A^+$, I-$E^+$), the latter expressing a second class II MHC molecule, I-$E^b$, due to a monomorphic $E\alpha^k$ transgene that pairs with the endogenous I-E$\beta^b$ chain. First, the naive T cell Vβ repertoire was altered in B6.$E^+$ thymi and spleens, potentially mediating different outcomes in T cell reactivity. Although the B6 and B6.$E^+$ responses to hen egg-white lysozyme (HEL) protein immunization remained similar, other immune models yielded differences. For viral infection, the quality of the T cell response was subtly altered, with diminished production of certain cytokines by B6.$E^+$ $CD4^+$ T cells. In alloreactivity, the B6.$E^+$ T cell response was significantly dampened. Finally, we observed markedly enhanced susceptibility to experimental autoimmune encephalomyelitis (EAE) in B6.$E^+$ mice. This correlated with decreased percentages of nTreg cells, supporting the concept of Tregs exhibiting differential susceptibility to negative selection. Altogether, our data suggest that expressing an additional class II MHC can produce diverse effects, with more severe autoimmunity providing a compelling explanation for limiting the expression of MHC molecules.



## 1. Introduction

The major histocompatibility complex (MHC) functions both in the periphery to present peptides to T cells and in the thymus to select the T cell repertoire (Jensen, 2007). Humans express three types of class I and three class II MHC molecules, while mice have up to three types of class I and only one or two class II MHC (Kelley et al., 2005; Kumanovics et al., 2003; Lawlor et al., 1990). It is a difficult process for thymocytes to make it past selection, and positive selection is particularly stringent with approximately 90% of thymocytes dying by neglect due to an inability to sufficiently bind (pMHC) complexes (Egerton et al., 1990; Jameson et al., 1995; Palmer, 2003; Scollay et al., 1980; Stritesky et al., 2013). Therefore, it is curious why vertebrates do not express more MHC molecules, which would theoretically result in an increase in the mature T cell population that successfully underwent selection as well as a greater diversity of the presented pathogenic peptide repertoire in the periphery. Indeed, mathematical modeling suggests that the number of MHC molecules can be increased significantly without suffering adverse effects on the ability to mount a response to pathogens, with estimates even as high as 1500 MHC molecules (Borghans et al., 2003; Nowak et al., 1992). The question of what impact expressing additional MHC molecules has on the immune response has intrigued immunologists over the years yet has not been answered thoroughly. Settling this issue is important for increasing our understanding of the factors influencing the nature of the T cell repertoire, which ultimately impacts a wide spectrum of immune responses including reactivity to pathogens, ability to tolerate transplantation, and susceptibility to autoimmune disease.

MHC molecules on medullary thymic epithelial cells and bone marrow derived antigen presenting cells (APCs) induce negative selection, deleting T cells containing high affinity T cell receptors (TCRs) to preserve self-tolerance (Gallegos and Bevan, 2004). This

*Abbreviations:* [$^3$H]TdR, tritiated thymidine; B6, C57BL/6; DN, double-negative; DP, double-positive; EAE, experimental autoimmune encephalomyelitis; HEL, hen egg-white lysozyme; LCMV, lymphocytic choriomeningitis virus; MOG, myelin oligodendrocyte glycoprotein; PPD, purified protein derivative; Treg, regulatory T cell.

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role implies that detrimental effects would ensue from increasing the number of MHC molecules, overly restricting the T cell repertoire and dampening anti-pathogen T cell responses. There is some evidence that the negative selection imparted by adding MHC molecules may be considerable. For instance, it was shown that the expression of I-E deletes the vast majority of Vβ17 TCR-expressing T cells (Kappler et al., 1987). Negative selection mediated by a single MHC molecule was demonstrated to account for the elimination of approximately 5% of thymocytes, and it seems likely that each additional MHC expressed would further decrease the T cell repertoire by that substantial percentage (van Meerwijk et al., 1997). And the fact that approximately 1–10% of T cells are alloreactive to non-self MHC molecules supports the notion that a significant portion of T cells would bind to a new MHC molecule encountered in the thymus, possibly too strongly (Bevan, 1977; Sherman and Chattopadhyay, 1993; Suchin et al., 2001). Negative selection by superantigen can also be a mechanism mediating the thymic deletion of particular Vβ TCR-expressing T cells. To elaborate, mouse mammary tumor virus superantigens bind regions on the MHC and TCR Vβ, stimulating massive T cell help for infected B cells and establishing infection. However, strains of mice that have integrated superantigen genes into their genome would delete T cells reactive to exogenous infection, a beneficial consequence that would explain the evolutionary preservation of superantigen-mediated elimination of a significant part of the TCR repertoire (Llewelyn and Cohen, 2002).

Knowing whether the introduction of additional MHC molecules results in either a net gain of positive selection or negative selection effects is integral in understanding the ultimate implications on immune responses. Thus far, there are conflicting reports on whether the expression of additional MHC molecules would be beneficial or detrimental to immune responses. MHC heterozygotes – obtained from crossing the F1 progeny of MHC-congenic mice – were shown to have greater pathogen resistance compared to their littermate MHC homozygote mice (McClelland et al., 2003; Penn et al., 2002). On the other hand, studies looking at the F1 progeny of parents with disparate MHC haplotypes have revealed a dampening of T cell reactivity in the context of viral infections. Both naive T cell precursor numbers and T cell cytokine production during infection with influenza and vaccinia viruses were shown to be negatively affected (Day et al., 2011; Flesch et al., 2010). The approach of looking at MHC heterozygote mice, however, is a complicated system that may result in heterogeneous responses in offspring within the same cross due to complex contributions from the varied MHC molecules (Moudgil et al., 1998). An alternative, simpler method is to investigate the effects of adding just one more MHC molecule. Studies employing this method focused on autoimmunity, with most concluding that expressing more MHC is beneficial. Adding I-E expression to the non-obese diabetic (NOD) background protected from diabetes, with I-E-mediated thymic deletion of pathogenic Vβ5 TCR-expressing T cells proposed to be a mechanism (Bill et al., 1988; Lund et al., 1990; Nishimoto et al., 1987; Reich et al., 1989). Expression of I-E on other backgrounds including H-$2^b$ and H-$2^q$ likewise resulted in protection from lupus, experimental autoimmune myasthenia gravis, and collagen-induced arthritis (Christadoss et al., 1990; Gonzalez-Gay et al., 1994; Merino et al., 1993). Yet there is a report that the addition of I-$E^b$ actually resulted in detrimental effects, enhancing susceptibility to disease in autoimmune thyroiditis (Brown et al., 2008). Having negative effects from the addition of a second MHC was also corroborated in a different model (viral infection) with a different MHC being investigated (I-A). Here, the $CD4^+$ T cell response to influenza peptides was diminished in mice expressing two I-A molecules compared to one (Nayak and Sant, 2012).

The I-E MHC, which has been a useful tool to add back to the mouse MHC repertoire, is puzzling for its disappearance from a substantial population of mouse strains. H-$2^b$, H-$2^s$, H-$2^f$, and H-$2^q$ haplotype containing mouse strains and approximately 20% of wild mice do not express I-E (Mengle-Gaw and McDevitt, 1985). Studies support the idea that there exists some advantage for losing I-E MHC expression. First, it was shown that three distinct mechanisms contribute to the loss of I-E expression (Mathis et al., 1983). Second, one of the mechanisms, a deletion in the *Eα* gene, was found to be identical between wild mice and H-$2^b$ plus H-$2^s$ haplotype mice, suggesting the mutation occurred early and disseminated widely throughout the mouse species (Dembic et al., 1985; Dembic et al., 1984; Tacchini-Cottier et al., 1995). However, it does not make sense why some mice still express both I-A and I-E class II MHC molecules and are capable of surviving normally if I-E is that harmful. Thus, we set out to resolve the important question of what impact does I-E have on immune responses. Although previous studies did reveal insight into the effect of expressing additional MHC molecules, including I-E, each focused only on one disease model. It is unknown whether adding a particular MHC exerts distinct effects on different T cell responses.

In our study, B6.$E^+$ mice, which have the monomorphic $E\alpha^k$ transgene thus enabling I-$E^b$ expression, were used to address the impact of having one additional class II MHC on a range of immune responses. We hypothesized that there would be negative consequences to immune responses from adding I-$E^b$, providing a reason for the limit on the number of MHC molecules expressed. Our results demonstrated diverse effects of I-$E^b$ across multiple immune models. In hen egg-white lysozyme (HEL) protein immunization, we found similar B6 and B6.$E^+$ responses. In viral infection with lymphocytic choriomeningitis virus (LCMV), I-$E^b$ elicited subtle differences in $CD4^+$ T cell cytokine production. In in vitro alloreactivity, there was decreased B6.$E^+$ T cell responses compared to B6. Finally, in an autoimmune disease model, experimental autoimmune encephalomyelitis (EAE), expression of I-$E^b$ resulted in detrimental consequences. There was significantly enhanced disease in B6.$E^+$ mice compared to B6. This was not attributable to greater numbers of pathogenic T cells or increased effector cytokine production. Instead, B6.$E^+$ mice had decreased regulatory T (Treg) cell percentages during EAE, a deficiency related to altered selection mediated by I-$E^b$. Altogether, the data suggest that the limitation on the number of MHC molecules we express may be to prevent autoimmunity.

## 2. Materials and methods

### 2.1. Mice

Balb/c, C57BL/6 (B6), and SJL mice were purchased from The Jackson Laboratory. B6.$E^+$ mice were the kind gift of Chella David (Mayo Clinic), and were derived from insertion of the monomorphic $E\alpha^k$ transgene into C57BL/6 × SJL embryos (Le Meur et al., 1985), which pairs with the endogenous I-E$\beta^b$ chain thus allowing the expression of I-$E^b$. The mice were extensively backcrossed with C57BL/6 mice onto the H-$2^b$ background, and this was confirmed by analysis of microsatellite markers at the Rheumatic Disease Core Center, Washington University School of Medicine (St. Louis, MO). Mice were bred and housed in specific pathogen-free conditions at the animal facility at the Washington University Medical Center (St. Louis, MO). All use of laboratory animals was approved and performed in accordance with the Washington University Division of Comparative Medicine guidelines.

### 2.2. Flow cytometry

Analysis of thymocytes and peripheral T cell populations was performed using anti-CD3 (145-2C11)-PE-Cy7, anti-CD25